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# Dioecy in *Wikstroemia pseudoretusa* (Thymelaeaceae) Endemic to the Bonin (Ogasawara) Islands

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The sexual mode of *Wikstroemia pseudoretusa*, endemic to the Bonin (Ogasawara) Islands, was examined and compared with that of *W. retusa*, distributed widely in the Ryukyu Islands, Taiwan and the Philippine Islands. Although the two species have generally been regarded as hermaphroditic, we found that *W. pseudoretusa* was actually dioecious and *W. retusa* was hermaphroditic. In plants of *W. pseudoretusa*, male flowers usually produced functional pollen grains, and their gynoecium was vestigial and lacked ovules, while female flowers had a functional gynoecium, including an ovule, and their anthers were vestigial and completely lacked pollen grains. Based on the morphological properties of these species and on the occurrence of hermaphroditism in a closely related *W. retusa*, distributed on the continental islands, the Bonin *W. pseudoretusa* seems to be an example of dioecy evolving from hermaphroditism within the Islands.

Key words: Bonin Islands, dioecy, oceanic islands, Ogasawara, pollen stainability, Thymelaeaceae, *Wikstroemia pseudoretusa, Wikstroemia retusa*

*Wikstroemia* Endl. is a genus comprising about 50 species, and is distributed from Southeastern Asia to India, and in northeast Australia and the Pacific islands (Peterson 1990). Only two of these species are known in Japan. One of them is *W. pseudoretusa* Koidz., endemic to the Bonin (Ogasawara) Islands, and the other is *W. retusa* A. Gray, distributed from the Ryukyu Islands to Taiwan, and in the Philippine Islands (Murata 1999). The two species have generally been regarded as hermaphroditic (Koidzumi 1919, Hamaya 1955, Murata 1999). However, throughout our preliminary studies we found that the plants of *W. pseudoretusa* occurring in the Chichi-jima and Haha-jima Islands of the greater Bonin Islands were sexually dioecious.

The Bonin Islands is one of the oceanic island groups that have never been connected to any landmass in geographical history (Asami 1970). On such oceanic islands, a relatively high percentage of dioecious species and their generic representatives have been noted (Carlquist 1974, Baker & Cox 1984, Kawakubo 1990, Sakai *et al.* 1995). In the Hawaiian Islands, it has been noted that most of the *Wikstroemia* species (12 spp.) are functionally dioecious and that their dioecy may have evolved from a hermaphroditism within the islands (Mayer 1990, Mayer & Charlesworth, 1992). Such an evolution to dioecy has also been reported for *Callicarpa* (Kawakubo 1990) and *Dendrocacalia* (Kato & Nagamasu 1995) in the Bonin Islands.

With that background, the species of concern here, *Wikstroemia pseudoretusa*, was presumed to be dioecious and to have evolved from a hermaphroditic species within the Bonin Islands. The species is generally regarded as being closely related to *W. retusa* (Koidzumi 1919, Toyoda 2003), although their phylogenetic relationship has not been confirmed. In this paper, we examined the sexual mode of *W. pseudoretusa* in detail to determine whether the species is truly dioecious, and further compared it with the sexual mode of *W. retusa*, occurring in the continental islands.

## Materials and Methods

*Wikstroemia pseudoretusa* and *W. retusa* usually grow in the scrubland of rocky ridges in the coastal areas, but the former sometimes occurs in the forest floor of the mountain areas (Shimizu, 1984). They usually flower from July to September. In the present study, we examined 47 individual plants of *W. pseudoretusa*, collected from two study sites, Nishikaigan and Amanoura-yama on Chichijima Island, the Bonin Islands (Tokyo Pref.), and compared them with 14 plants of *W. retusa*, collected from Nobaru on Iriomote Island, the Ryukyu Islands (Okinawa Pref.).

To observe sexual and morphological differentiation, several flowers were randomly collected from the plants growing in the study sites and preserved in 50% or 70% ethanol. The sexual expression of the plants was examined by recording the presence or absence of pollen grains and ovules. Pollen stainability of more than 300 grains per flower was examined using aniline blue in lactophenol. Gynoecia were sectioned longitudinally to ascertain female fertility. For evaluating pollen stainability (male fertility) and female fertility, three flowers per plant were examined. Voucher specimens will be deposited in Makino Herbarium (MAK), Tokyo Metropolitan University, Tokyo.

For scanning electron microscopy (SEM), flowers were dehydrated in an ethanol:t-butanol series. Dehydrated materials were dried using a freeze-drying device (JFD-300, JEOL), mounted onto SEM stubs using double-sided carbon tape, coated with gold using an ion sputter (JFC-110E, JEOL) and observed using a scanning electron microscope (JSM-5600LV, JEOL).

## Observations and Discussion

As in other species of *Wikstroemia* (Peterson 1990, Heber 2003), the two Japanese species, *W. pseudoretusa* and *W. retusa*, possess yellow to yellowish and apetalous flowers, and their flowers are composed of a tubular calyx with eight stamens, an uniovulate gynoecium and a disk with a few lobes (Fig. 1). Anthers are inserted at the distal part of the floral tube and arranged in two whorls, and the gynoecium with a globose stigma is always positioned below the anthers.

Throughout our observations of floral morphology, pollen stainability and female fertility, we found that the sexual expression of the examined flowers was uniform within a plant. *Wikstroemia pseudoretusa* in the Bonin Islands was dioecious while *W. retusa* in the Ryukyu Islands was hermaphroditic, although the former species has previously been described as hermaphroditic (Koidzumi 1919, Hamaya 1955, Murata 1999).

In *Wikstroemia pseudoretusa*, both male and female flowers produce nectar and possess anthers. The anthers of female flowers were slightly smaller than those of male flowers, and completely lacked pollen grains, while the anthers of male flowers produced a large number of pollen grains. As shown in Figure 2, pollen stainability of the male plants

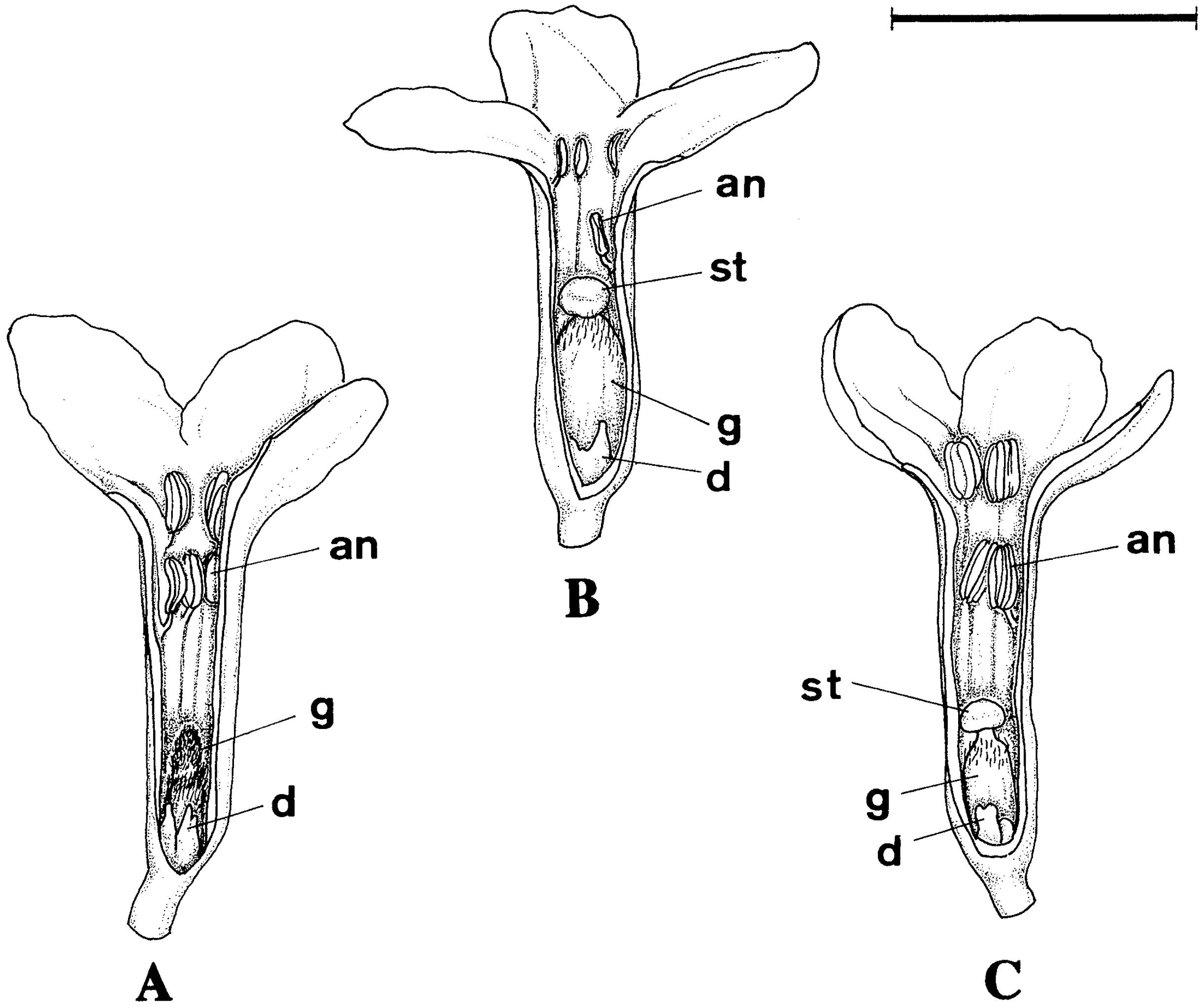

FIG. 1. Flowers of Japanese *Wikstroemeria*. A, Male flower of *W. pseudoretusa*. Gynoecium is abortive and a stigma is not found. B, Female flower of *W. pseudoretusa*. Anthers are vestigial and pollen grains are absent. C, Hermaphroditic flower of *W. retusa*. an, anther; d, disk; g, gynoecium; st, stigma. Scale bar represents 5 mm.

from the two study sites was usually high and greater than 80%. However, the pollen stainability in a few plants collected from Nishikaigan was less than 10% (Fig. 2A). These plants, despite the remarkably lower pollen stainability, were regarded as being essentially male, since they had no stigma and no ovule, as described below. In the female flowers, the gynoecium possesses a globose stigma terminally, while in the male flowers it was abortive in appearance and usually lacked stigmas. Figure 3 shows the scanning electron micrographs of gynoecia and their longitudinal views in *W. pseudoretusa*. It is clear from this figure that the female flowers produce an ovule within the ovary (Fig. 3A, A'), while the male flowers usually lack a stigma and produce no ovule (Fig. 3C, C'). In some male flowers, the gynoecium had a smaller stigma, but completely lacked ovules in the ovary (Fig. 3B, B').

In contrast, all of the *Wikstroemia retusa* plants examined had only hermaphroditic flowers that produce pollen grains, and an ovule within the ovary (Figs. 1 & 4). Pollen stainability obtained from 14 plants ranged from 65% to 96% (Fig. 2C).

In earlier studies, a relatively high percentage of dioecious species has been reported for the floras

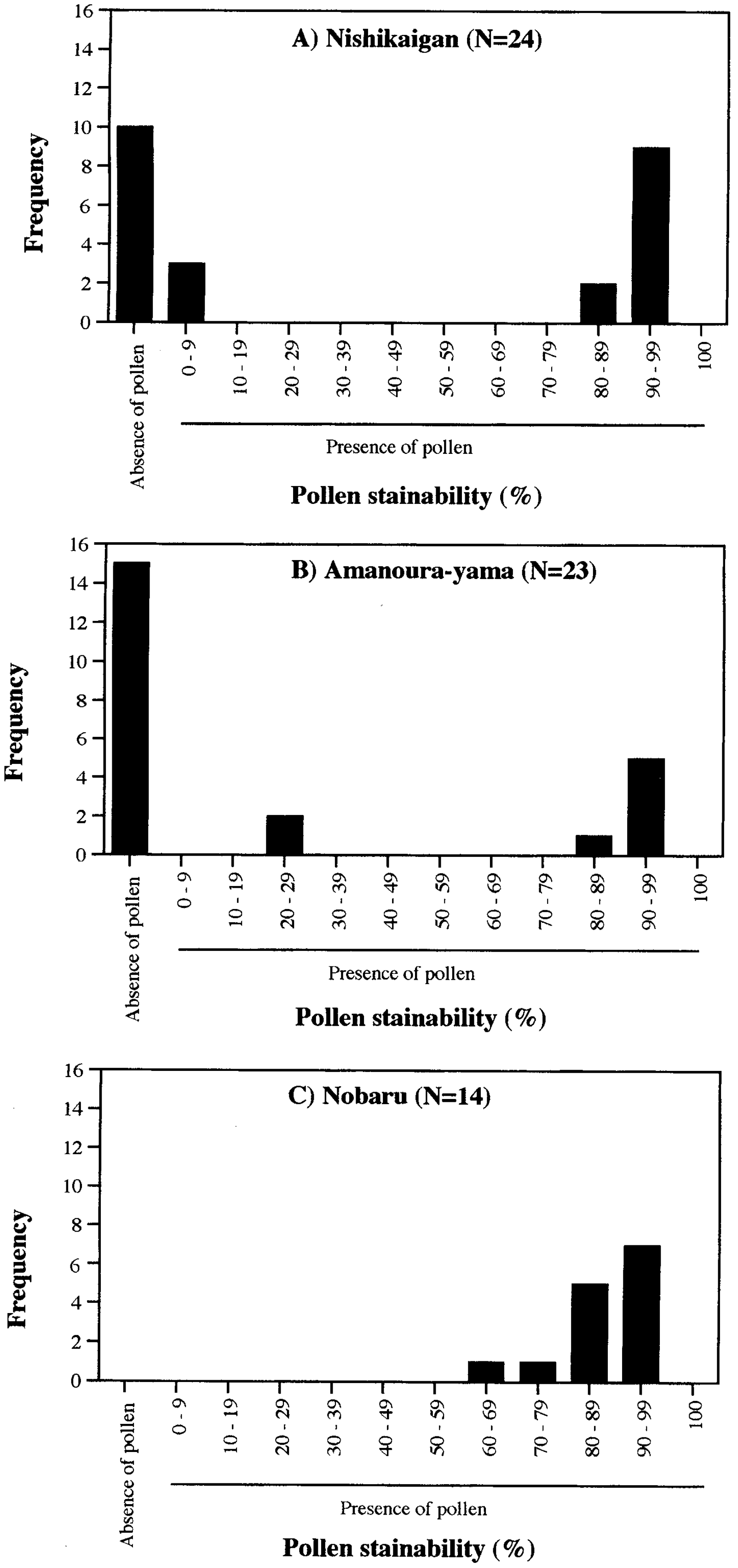

FIG. 2. Frequency distribution of pollen stainabilities of *Wikstroemia pseudoretusa* from two sites (A and B) and of *W. retusa* from one site (C). Values are based on individual means from examinations of three flowers per individual. Frequency represents the number of individuals. Note that the frequency of individuals lacking pollen grains (female frequency) is also shown on the

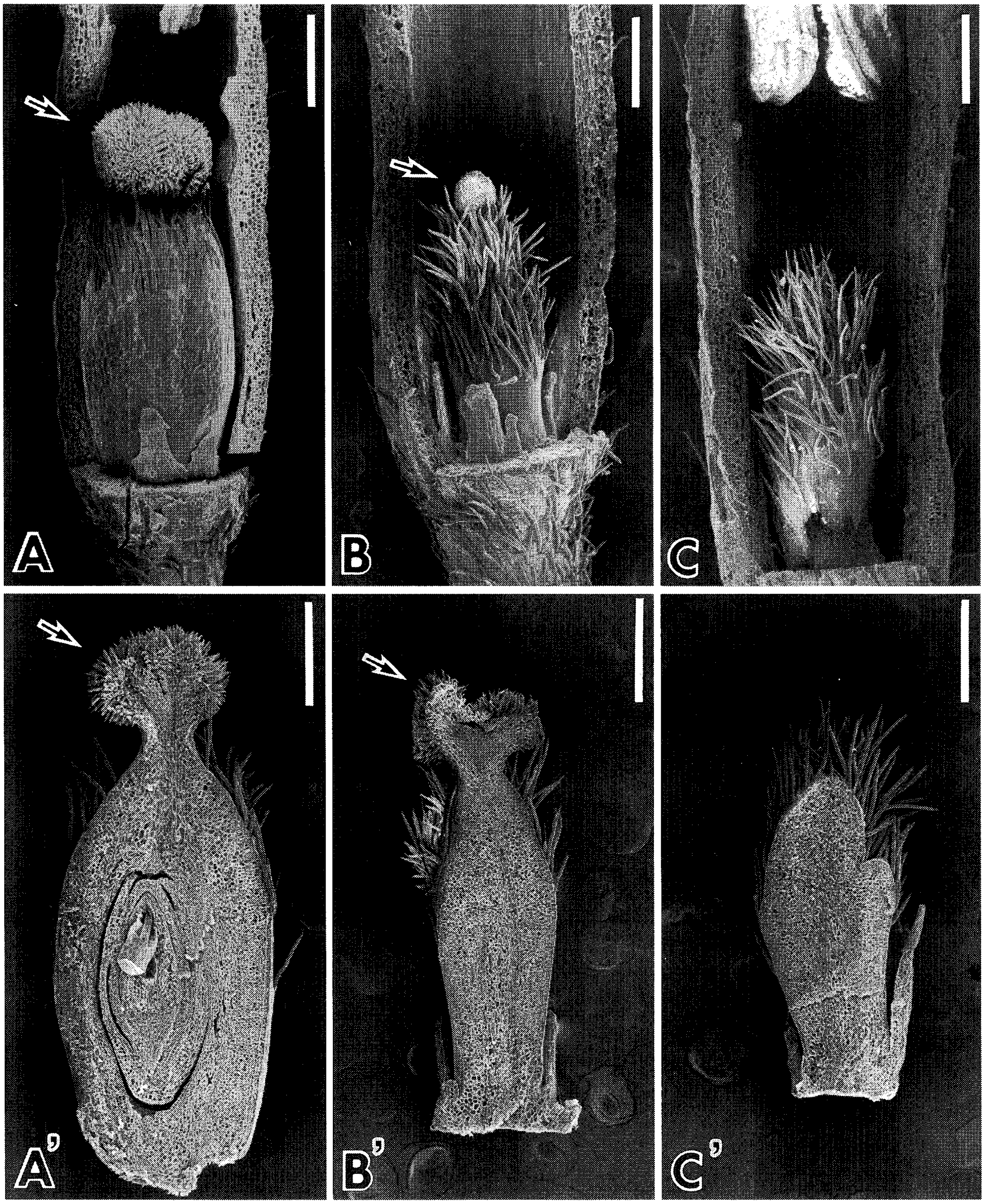

FIG. 3. Scanning electron micrographs of gynoecia and their longitudinal sections in *Wikstroemia pseudoretusa*. A & A', female flower in which one ovule is obvious. B & B', male flower in which gynoecium possesses a small stigma but lacks an ovule. C & C', male flower in which the stigma and ovule are absent. Arrows indicate stigmas. All scale bars represent 500 μm.

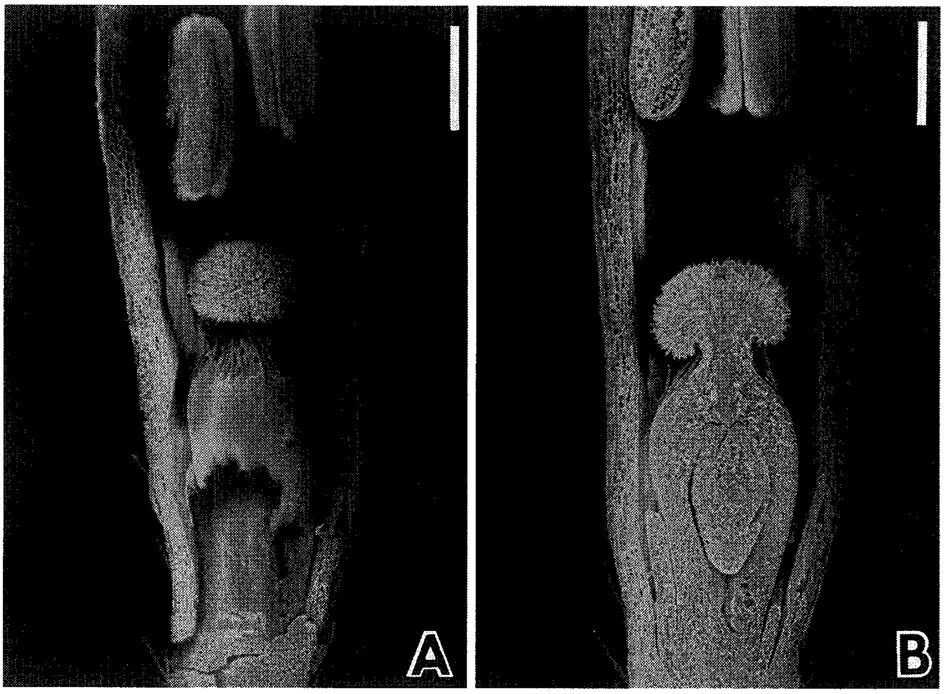

FIG. 4. Scanning electron micrograph of a gynoecium (A) and its longitudinal section (B) in *Wikstroemia retusa*. Scale bars represent 500 μm.

of oceanic islands, such as the Hawaiian Islands (14.7%, Sakai *et al.* 1995), Tonga (16%, Baker & Cox 1984), Guam (13%, Baker & Cox 1984) and the Bonin Islands (9.7%, Kawakubo 1990). However, it has been noted that some genera are dioecious on oceanic islands but hermaphroditic on continents, including *Ilex* (Aquifoliaceae), *Labordia* (Loganiaceae), *Pittosporum* (Pittosporaceae), *Bobea* (Rubiaceae), *Hedyotis* (Rubiaceae), *Straussia* (Rubiaceae), *Zanthoxylum* (Rutaceae), *Santalum* (Santalaceae) and *Wikstroemia* (Thymelaeaceae) on the Hawaii Islands (Carlquist 1974, Sakai *et al.* 1995), and *Callicarpa* (Kawakubo 1990) and *Dendrocacalia* (Kato & Nagamasu 1995) on the Bonin Islands. For the genus *Wikstroemia*, it has been reported that the Hawaiian species are almost entirely functionally dioecious, and it has been proposed that their sexual mode evolved within the islands (Mayer 1991; Mayer & Charlesworth 1992).

As described above, *Wikstroemia pseudoretusa* in the Bonin (Ogasawara) Islands was found to be dioecious, and *W. retusa* from the Ryukyu Islands was found to be hermaphroditic. It is also known that all *Wikstroemia* species outside of the Hawaiian Islands are hermaphroditic (Mayer 1991; Mayer & Charlesworth 1992), except for one unnamed dioecious species from New Caledonia (Skottsberg 1972). In *W. pseudoretusa*, the female flowers possess vestigial anthers that produce no pollen grains, and the male flowers sometimes retain the gynoecium with a small stigma vestigially. Based on these morphological properties and on the occurrence of hermaphroditism in *W. retusa*, the Bonin *W. pseudoretusa* seems to be an example of dioecism evolving from hermaphroditism within the Bonin Islands, as in the Hawaiian Islands, although further evolutionary studies are needed for these species.

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